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The Role of Some Promising Biomarkers in Diagnosis Neonatal Sepsis Caused by Multidrug Resistance *Acinetobacterbaumannii*

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Abstract

A total 120 newborn were studied from Neonatal Care Unit / Children Welfare Teaching Hospital /Medical city / Baghdad / Iraq , from November 2019 to end of February 2020, Male were 77 (64.17%) and 43 (35.83%) female, age ranged between 1 day to 27 days. 5 ml of Blood specimen collected from patients , 2.5 – 3.0 ml inject immediately in Brain heart infusion and incubated 24 hours at 37°C before cultured on different media (MacConkey agar and Blood agar) , after the growth of bacteria, the isolates were identified by microscopic examination , biochemical tests , Analytical profile index (API 20E) and VITEK -2 system . Another specimen (1 ml) were taken for screening level of Neutrophil Cluster of differentiation (nCD64) , by ELISA technique, ELISA technique was used to detect the level of expression of nCD64. The result of present study revealed the level of nCD64 in neonatal sepsis (*A. baumannii*+ve) group (28.86 ± 1.84) was significantly higher than neonatal sepsis (*A. baumannii*-ve) group (14.76 ± 2.16) and control (2.33 ± 0.58) ($P \leq 0.01$).

Keywords:*Acinetobacterbaumannii* , neonatal sepsis , n CD64

Introduction

Neonatal sepsis refers to an infection involving bloodstream in newborn infants less than 28 days old. It continues to remain a leading cause of morbidity and mortality among infants, especially in middle and lower-income countries [1] . It is the 20% of neonates develop sepsis and approximately 1% death due to sepsis [23]. The organisms that give neonatal sepsis vary over time and change from region to region, it can even alteration from hospital to hospital [4].Neonatal sepsis is divided into 2 groups based on the time of presentation after birth: Early-onset sepsis (EOS) and Late-onset sepsis (LOS) . EOS refers to sepsis in neonates at or before 72 hours of life, and LOS is defined as sepsis occurring at or after 72 hours of life. Although, some experts use 7 days as the cutoff date [567], Early-onset sepsis (EOS) is generally caused by the transmission of pathogens from the mother genitourinary system to the newborn or the fetus. These pathogens can ascend the vagina, the cervix, and the uterus, and can also infect the amniotic fluid. Neonates can become infected in utero or during delivery as they pass through the vaginal canal. Late-onset sepsis (LOS) usually occurs by the transmission of pathogens from the environment after delivery, such as contact from healthcare workers or caregivers. LOS may also be caused by a late manifestation of vertically transmitted infection. Infants that require intravascular catheter insertion, or other invasive procedure that disrupts the mucosa, are at increased risk for developing LOS. Preterm neonates are at higher risk for sepsis/infection than term neonates, as they tend to require more invasive procedures than term neonates. [8]*Acinetobacterbaumannii* are aerobic Gram-negative, catalase-positive, oxidase-negative coccobacilli which have the ability to survive in the hospital environment for prolonged periods

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[9]Neonates admitted to Neonatal Intensive Care Unit (NICU) are at increased risk of contracting HAIs due to their immature immune system and frequent invasive manipulations. Blood stream infections (BSIs) caused by A. baumannii occur mainly in premature low-birth-weight infants. [10] Early diagnosis of sepsis is a challenge. Blood culture results are not available for 48 hours; several biomarkers are available for early diagnosis of sepsis. Of course, early sepsis detection could reduce mortality and hospital stays [11].Among these markers, is nCD64, it is high-affinity immunoglobulin Fc γ receptor I, is constitutively expressed on monocytes and to a very low extent on resting neutrophils. Interestingly, CD64 expression on neutrophils up regulated within six hours after their activation. CD64 expression on neutrophils increases once these become activated by the proinflammatory cytokines interferon gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF), produced in response to infection or after exposure to endotoxin [1213]

Patient and method

This work was performed on 120 patients referred to Neonatal Care Unit (NCU) / Children Welfare Teaching Hospital /Medical city / Baghdad / Iraq. Selection of the patients was accomplished with assistance of pediatrician in the hospital. The study was carried out on 120 patients suspected septicemia (77 male and 43 female) ranged between 1 day and 27 days. Data were collected through direct interview with the patient's parents and by seeking his / her hospital record as well as previous medical report.One hundred and twenty (120) samples collected from Neonatal Care Unit (NCU)/ Children Welfare Teaching Hospital /Medical city / Baghdad , during the period from November 2019 to end of February 2020. Samples were collected in a sterile tubes containing nutrient broth from patients.ELISA technique Blood sample must be centrifuged for 15 min. taken serum for screened level of nCD64, by ELISA technique, To assess the markers for their early diagnosis neonatal sepsis.

Results and Discussion

Out of the total 120 newborn babies suspected as sepsis were studied in Neonatal Care Unite /Children Welfare Teaching Hospital /Medical city / Baghdad / Iraq during the period from November 2019 to end of February 2020 . 77 (64.17%) were male and 43 (35.83%) female; age ranged between 1 day to 27 days.Eighty seven 87 (72.5 %) neonates diagnosed with sepsis as confirmed by blood culture results which of 64 (53.33 %) Gram negative , 23 (19.16%) Gram positive and no bacterial growth occurred in 33 (27.5%) samples . (Figure 1) , Among these , 29 (33.3%) had A. baumannii bacteremia , whereas 58 (66.7%) with other bacteria (Table 1).

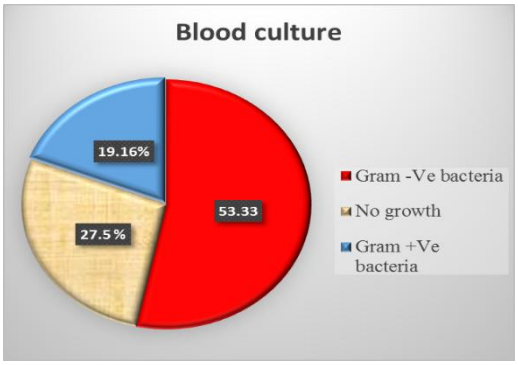

Figure (1): The

frequency of culture positive and negative cases among study specimens

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Table (1): The percentage of A.baumannii infection in neonates with septicemia .

Types of isolates	Neonates with septicemia
A. baumannii	29 (33.3%)
Other bacteria	58 (66.7%)

Identification of the isolates by Biochemical tests.

All isolates showed negative results for oxidase test, motility test, indole production test and urease production test, while the isolates gave positive results to catalase test and citrate utilization test. Kligler iron agar developed an alkaline slant, no change bottom, H2S negative without gas production (Table 2)

Table 2 : Biochemical test results for *A. baumannii*.

Biochemical tests	Results
Catalase production	**+**
Citrate utilization	**+**
Hemolysin production	**-**
Indole production	**-**
Lactose fermentation	**-**
Motility	**-**
Oxidase production	**-**
Kliglar iron agar (KIA)	**Alkaline slant / No change bottom, No gas , No H2S**
Urease production	**-**

(+) positive result, (-) negative result

Comparison between the expressions of nCD64 in healthy subjects, neonatal sepsis (Acineto. +), neonatal sepsis (Acineto -).

Table (3) shows the level of nCD64 in neonatal sepsis (A. baumannii +ve) group (28.86 ± 1.84) was significantly higher than neonatal sepsis (A. baumannii -ve) group (14.76 ± 2.16) and control (2.33 ± 0.58) ($P \leq 0.01$).

Table (3): Comparison between difference groups in n CD64 conc.

Group	Mean ± SE of n CD64
G1: Acineto	**28.86 ± 1.84 a**
G2: Other bacteria	**14.76 ± 2.16 b**
G3: Control	**2.33 ± 0.58 c**
LSD value	**4.975 ****

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P-value	**0.0001**
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

Distribution of Organisms according positive and negative culture in study group .

The incidence and microbiology of neonatal sepsis varies worldwide. Blood culture has been regarded as the gold standard for the confirmation of sepsis. In the present study , 120 Blood sample taken for culture from neonate which admitted in Neonate Care Unite . The result revealed out of 120 sample , positive culture 87 (72.5%) higher than negative culture 33 (27.5%) , the results of this study is confirmed with the previous reports studies [14] .reported that positive culture more than negative culture 71.8% of neonatal blood stream infections in India were caused by Gram-negative bacteria, with Klebsiellaspp accounting for 16.4%, Pseudomonas spp. 13.6%, E. coli 11.8%, Enterobacter spp. 11.4% and Acinetobacter spp.10% , Another study [15] demonstrated that Gram-negative bacteria were more frequent than gram positive bacteria in Pakistan with a frequency of 54.6% and 45.4% respectively ,this agrees with the study of [16] who revealed that out of 158 neonates yielded positive results in 57% of cases, while the remaining 43% gave negative results. A similar result was reported by Ako-Nai et al (1999)[17] in Nigeria with a positive percentage of 55% . Slightly higher positive results were reported by Rohsiswatmo(2006) [18]in Indonesia (65.3%), Rahman et al(2002) .[19] in Pakistan (62.8%) and by Macharashvili et al (2009)[20] in Georgia (63%) and explained that the Gram negative organisms were the most common causative agents of bacterial sepsis and a significant cause of mortality and morbidity in the newborn. In contrast, much lower positive results were reported from Iran (5.6%)[21] , Kuwait(8.7%) [22] and Saudi Arabia (5%)[23]These variations can be attributed to many different factors of which antibiotic therapy prior to the laboratory diagnosis may have had the most important influence on the low culture results.

Expression of nCD64 in all study subjects .

In current study showed the level of nCD64 in neonatal sepsis with A. baumannii (28.86 ± 1.84) was significantly higher than neonatal sepsis without A. baumannii (other bacteria) (14.76 ± 2.16) and control (2.33 ± 0.58) (P≤0.01). There is increasing evidence in the literature to sup¬port the use of nCD64 for diagnosing sepsis in general as well as neonatal sepsis, van der Meer et al (2007)[24] they provide CD64 expression on the cell surface of PMN and monocytes is considered to be a very early step of the immune host response to bacterial infection, another study that revealed nCD64 good diagnostic marker for neonatal sepsis [25], CD64 is continuously expressed at a very low level on the surface of neutrophil leukocytes , in absence of bacterial stimulation. During bacterial sepsis the expression of CD64 is significantly increased [26]

Conclusions

Acinetobacterbaumannii is one of the emerging cause of nosocomial infection in NCU , due to develop antibiotic resistance. Gram negative bacteria were found to be commonest cause of neonatal septicemia. A significant increase of nCD64 presence of *Acinetobacterbaumannii* compared in patients with other bacteria and healthy subjects. nCD64 can also help clinicians the clinical course of neonatal sepsis.

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